Hypoglycemia and Reduced Feed Intake in Broiler Chickens Treated with Metformin

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ABSTRACT The bi-guanide metformin is used to treat noninsulin dependent diabetes in obese patients. In addition to having antihyperglycemic effects, metformin is also anorectic and reduces BW. These studies were performed to determine if metformin possesses similar properties in chickens. Metformin-HCl was administered to 14-day-old broiler chickens at either 300 or 600 mg/kg per day in the drinking water for 10 d while monitoring BW and feed intake. No changes in water intake were observed, while feed intake and daily gains were only significantly reduced by the 600 mg/kg dose. After oral administration of a single dose of 300 mg/kg metformin-HCl, feed intake was significantly reduced by 4 h and remained suppressed for greater than 24 h relative to controls. Plasma hormones and metabolites (glucose, lactate, insulin, glucagon, uric acid, nonesterified fatty acid, and triglycerides) were monitored at 1, 2, 3, 6, and 24 h posttreatment. Significant and acute decreases in blood glucose, insulin, and triglycerides were observed at 3 h posttreatment as compared to controls. Opposing acute increases in glucagon and NEFA levels were also observed at 3 h followed by an increase in uric acid 6 h posttreatment. These observations suggest that metformin induces metabolic changes in birds, similar to that observed in mammals and may act in a common manner. Metformin-HCl may be useful in glucose metabolism studies by inducing hypoglycemia, a condition rarely observed in birds.

(*Key words*: metformin, appetite, hypoglycemia, insulin, glucagons)

2003 Poultry Science 82:106–110

INTRODUCTION

Metformin-HCl (Glucophage, Bristol-Meyers Squibb) is regarded as the first choice for therapy of type 2 diabetes that is generally characterized by insulin resistance or specifically obese patients with hyperinsulinaemia (Bray, 1999; Cusi and DeFronzo, 1998). By reducing blood glucose without inducing hypoglycemia and further hyperinsulinaemia, as well as reducing feed intake, metformin-HCl provides a suitable antidiabetic treatment (Lee and Morley, 1998; Rouru et al., 1992). Complications can occur with metformin-HCl induction of lactic acidosis (Stumvoll et al., 1995). High levels of fasting blood glucose, insulin resistance, and obesity are not just hallmarks of type 2 diabetes but are also normal physiologic conditions for chickens (Simon, 1989).

The regulation of feed intake in birds occurs both centrally and peripherally, similar to mammals (Denbow, 1989; 1994). The peripheral regulation of feed intake has been described for effects of glucose, lipid, and amino acids. Glucose administered by intrahepatic infusion has been shown in several studies to decrease feed intake in various chicken lines. Similar infusions into the jugular vein showed no effects on feed intake suggesting the site of action was the liver (Shurlock and Forbes, 1981; Lacy et al., 1985). Similar infusions of specific lipid species had similar effects on feed intake in chickens with the liver indicated as the site of action (Lacy et al., 1986; Denbow et al., 1992). The response of feed intake to amino acid infusions has been shown to be controlled centrally and can be both inductive and repressive as demonstrated by Tobin and Boorman (1979), as well as others (Shurlock and Forbes, 1984; Rusby and Forbes, 1987; Lacy et al., 1986). The effect of specific hormones has also been investigated for effects on feed intake and metabolism. Chickens have been generally characterized as being insulin insensitive (Simon, 1989). Metabolic response to insulin treatment requires significantly high dosages. One hormone that appears to exert greater influence over feed intake and energy metabolism in the chicken is glucagon (Hazelwood, 1984). Glucagon infusion decreases feed intake without dependence on the infusion site (Smith and Bright-Taylor, 1974). The effect of glucagon to reduce feed intake can be blocked by vagotamy, suggesting that its

Received for publication Janurary 3, 2002.

Accepted for publication September 18, 2002.

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Abbreviation Key: AMPK = adenosine monophosphate-activated protein kinase.

effects are to alter hepatic metabolism, which signals the brain via the vagus resulting in altered appetite. The effect of glucagon treatment has little impact on circulating glucose levels (Howes and Forbes, 1987).

These studies were undertaken to determine the effects of metformin-HCl on avian metabolism and feed intake. Various treatment regimes were undertaken and multiple metabolic factors monitored during experimental periods to access the specific action of metformin-HCl in birds. It would be advantageous to have the ability to manipulate feed intake in broiler breeder chickens where obesity-related reduction in reproductive efficiency has required the implementation of feed restriction regimes.

MATERIALS AND METHODS

Animal Experiments

Male broiler chicks were purchased from Shaver Poultry at one d of age.[2] Birds were housed in brooder batteries in a climate-controlled facility and maintained on freely available water and feed consisting of a commercial corn/soy diet at 21% CP and 2,900 kcal ME/kg.

Experiment 1. Broiler chicks (n = 32) randomly assigned to 8 pens were fed ad libitum to 28 d of age. At Day 7, metformin-HCl was introduced in the water at a dose of 300 mg/kg per day in 4 of 8 pens.[3] Water intake was monitored each day for each pen. The concentration of metformin-HCl in the water was adjusted daily and was based on the previous 24-h water intake level for administration of 300 or 600 mg/kg per day. As the birds grew, increases in daily water intake result in slightly greater than the administered dose of metformin-HCl. At Day 16, the metformin-HCl dose was increased to 600 mg/kg per day. Feed intake, water intake, and BW were monitored from Days 7 to 28. At Day 28, blood was collected from the wing vein for subsequent analysis.

Experiment 2. Broiler chicks (n = 48) randomly assigned to eight pens were fed ad libitum to 21 d of age. At Day 21, four pens (4 birds each) were treated with either metformin-HCl (300 mg/kg per day) or water (vehicle) by intraesophageal administration. Blood samples were collected from the wing vein over a time course of 0, 1, 3, 6, 24, and 48 h posttreatment (n = 4). Also, over the course of the experiment, feed intake, water intake, and BW were recorded.

Plasma Chemistries and RIA

Blood samples were collected in EDTA-treated tubes, centrifuged at 2000 × *g* for 20 min and plasma transferred to tubes containing 1,000 IU Trasylol preservative.[4] Plasma glucose and lactate were determined by specific electrode analysis.[5] Double antibody radioimmunoassay was used to determine plasma concentrations of insulin, intraassay coefficient of variation of 2.9% (McMurtry et al, 1983). Plasma glucagon was determined using an RIA kit.[6] Nonesterified fatty acid assays were performed by specific kit.[7] Triglycerides and uric acid levels were also determined using specific kits.[8]

Statistics

The data were analyzed by either Students *t*-test, two-way analysis of variance, or Dunnett's test using the JMP statistical discovery package (2000) for effects by treatment or time or both. Data are presented as the mean ± standard error. Significant differences in data groups ($P < 0.05$) were determined.

RESULTS AND DISCUSSION

Chronic treatment with metformin-HCl orally in drinking water at 300 or 600 mg/kg per day had little apparent effect on the measured plasma hormones and metabolites with the exception of glucagon, which was significantly increased (Table 1). During the study period there was no effect of treatment on water intake. The monitoring of feed intake and average daily BW gain showed significant effects of the 600 mg/kg per day dosage of metformin-HCl from Days 18 through 28 of treatment (Figure 1). This difference in feed intake resulted in a decrease of approximately 15% of BW by the end of the 28-d period as a result of metformin-HCl treatment. This reduction in feed intake was encouraging for the efficacy of metformin-HCl as a modulator of appetite in chickens. It was not apparent that the differences in feeding behavior were associated with any other behavioral change that may have limited intake.

Acute metformin-HCl treatment and subsequent monitoring, describes more thoroughly the metabolic impact than the results obtained during chronic metformin-HCl treatment. After treatment with either 300 mg/kg of metformin-HCl or vehicle (water) by intraesophageal administration, feed intake, and multiple hormones and metabolic factors were monitored for a period of 48 h. The hormone and metabolite data are summarized in Table 2. Significant effects on feed intake were observed after a period of 4 h and persisted for at least 24 h posttreatment (Table 2). This change in feed intake was not accompanied by any change in water intake or apparent change in behavior.

Dramatic metabolic changes occurred within the first 3 h posttreatment. By 3 h significant decreases in glucose, insulin, and triglycerides occurred (Table 2). Significant increases in glucagon, lactate, and nonesterified fatty acid occurred simultaneously. This acute phase is followed by an increase in uric acid at 6 h posttreatment. These deviations return to control levels by 24 h posttreatment with the exception of lactate, which remained increased

[2]Shaver Poultry, Cambridge, Ontario, Canada.
[3]D5035, Sigma Chemical Co., St. Louis, MO.
[4]Bayer Corporation, West Haven, CT.
[5]YSI Biochemistry Analyzer, model 2700 Select, YSI, Yellow Springs, OH.
[6]GL-32K Linco Research, Inc., St. Charles, MO.
[7]994.75409, Wako Chemical, Richmond, VA.
[8]686-A and 337-A, Sigma Chemical Co., St. Louis, MO.

TABLE 1. Chronic effects of metformin effects on hormones and metabolites[1]

Measurement	Control	Metformin	*P*-value
Glucose (mg/L)	1,561 ± 261	1,597 ± 276	NS
Lactate (mg/L)	269 ± 93	265 ± 71	NS
Insulin (ng/mL)	3.15 ± 1.7	2.68 ± 0.74	NS
Glucagon (pg/mL)	206 ± 18	277 ± 30	0.007

[1]Values are presented as the mean ± standard error. Differences were determined by *t*-test. NS = $P > 0.05$ (n = 16).

until 48 h posttreatment. By 3 h definitive response to metformin-HCl treatment is observed, either as a response to metformin-HCl directly or to metformin's action on a particular hormone or metabolite. The significance of these effects by treatment and time, and the interaction of treatment and time are shown by two-way ANOVA in Table 2.

Previous hypoglycemic clamp studies indicate that metformin-HCl does not interfere with the normal hormonal and metabolic response to hypoglycemia (Fruehwald-Schultes et al., 2001). The response of insulin and glucagon to the change in blood glucose is to signal for glucose release either by glycogenolytic or gluconeogenic pathways. The resulting high levels of glucagon may be responsible for observed effects on feed intake (Hazelwood, 1984). The response of simultaneous reduction of triglycerides and increase in NEFA also indicate a response to glucose depression by shifting to alternative energy sources in the form of lipid. Similar increases in lactate and uric acid also suggest the search for alternative energy stores in the form of muscle glycogen and amino acid catabolism.

The action of metformin-HCl to reduce feed intake without the induction of hypoglycemia in humans is not completely shared with birds. Birds appear to be acutely sensitive to metformin-HCl, which induces hypoglycemia, an effect it rarely has in humans (Wiernsperger and Bailey, 1999). The ability of the birds to tolerate a 50% reduction in blood glucose levels without exhibiting any apparent behavioral changes was quite surprising. Induction of hypoglycemia in chickens has historically been difficult to achieve (Simon, 1989). Recent studies attempting to identify the molecular mechanism of action of metformin-HCl have targeted both manipulation of glucose and lipid metabolism in the liver (Zander et al., 2001). One study suggests that metformin-HCl increases

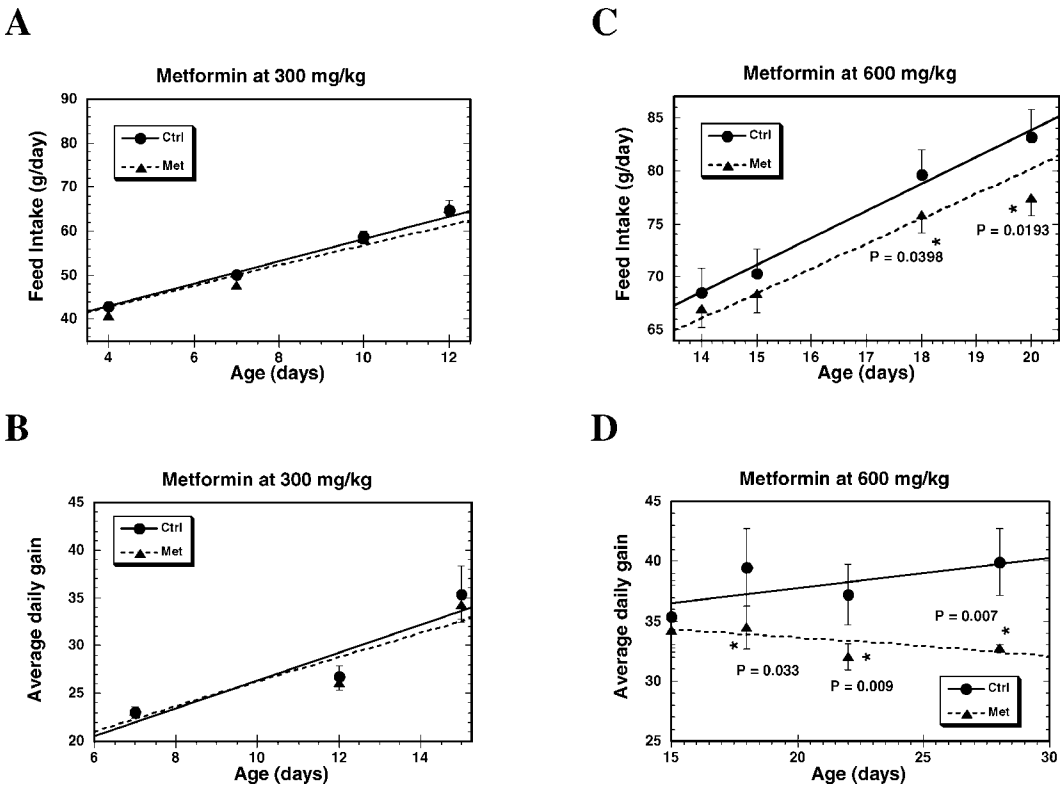

FIGURE 1. Chronic effects of metformin on feed intake and average daily gain. Oral administration of metformin at 300 mg/kg per day had no effect on intake (Panel A) or gain (Panel B). Dosing at 600 mg/kg per day had significant effects on both intake (Panel C) and gain (Panel D). Significant differences at sampling points are indicated by the inclusion of the respective *P*-values (n = 4). Trend lines shown were generated by linear regression to all data points at a given sampling time using Kaleidagraph 3.0 (Synergy Software, Reading, PA).

TABLE 2. Acute effects of metformin on feed intake, hormones, and metabolites

Measurement	Time (hours)	Control	Metformin	ANOVA *P*-value
Feed intake (g/bird per h)	0	3.50 ± 0.35		Treatment = 00220
	4	3.62 ± 0.63	2.14 ± 0.50	Time = 0.0487
	24	2.53 ± 0.47	1.57 ± 0.24*	Treatment × time = 0.0331
	48	3.77 ± 0.43	3.47 ± 0.39	
Glucose (mg/L)	0	2,550 ± 72		Treatment = 0.0015
	1	2,385 ± 50	2,221 ± 50	Time = NS
	3	2,634 ± 132	1,177 ± 132*	Treatment × time = 0.0085
	6	2,435 ± 202	2,124 ± 202	
	24	2,393 ± 159	2,574 ± 159	
	48	2,577 ± 76	2,593 ± 76	
Lactate (mg/L)	0	611 ± 114		Treatment = 0.0069
	1	558 ± 42	572 ± 50	Time = NS
	3	474 ± 39	766 ± 39	Treatment × time = NS
	6	659 ± 95	781 ± 95	
	24	421 ± 48	625 ± 48	
	48	508 ± 48	526 ± 48	
Insulin (ng/mL)	0	4.92 ± 0.76		Treatment = 0.0420
	1	4.15 ± 0.41	4.04 ± 0.41	Time = NS
	3	5.83 ± 0.31	2.24 ± 0.31*	Treatment × time = 0.0044
	6	4.56 ± 0.92	3.69 ± 0.92	
	24	9.97 ± 1.52	9.75 ± 1.52	
	48	4.31 ± 0.38	5.62 ± 0.38	
Glucagon (pg/mL)	0	112 ± 12		Treatment = 0.0018
	1	109 ± 18	146 ± 18	Time = NS
	3	110 ± 43	1,061 ± 43*	Treatment × time = 0.0463
	6	135 ± 79	275 ± 79	
	24	125 ± 27	130 ± 27	
	48	146 ± 28	183 ± 28	
NEFA (μEq/L)	0	206 ± 9.8		Treatment = 0.0014
	1	199 ± 10	222 ± 10	Time = NS
	3	188 ± 72	1,636 ± 72*	Treatment × time = 0.0292
	6	202 ± 123	489 ± 123*	
	24	169 ± 32	214 ± 32	
	48	168 ± 14	163 ± 14	
Triglycerides (mg/dL)	0	8.99 ± 0.88		Treatment = 0.0374
	1	7.94 ± 0.44	9.15 ± 0.44	Time = NS
	3	10.20 ± 0.75	4.21 ± 0.75	Treatment × time = NS
	6	6.66 ± 1.3	5.83 ± 1.3	
	24	9.97 ± 1.5	9.75 ± 1.5	
	48	9.95 ± 0.80	8.42 ± 0.80	
Uric acid (mg/dL)	0	6.59 ± 0.53		Treatment = 0.0331
	1	5.67 ± 0.84	6.15 ± 0.84	Time = NS
	3	6.70 ± 0.99	8.63 ± 0.99	Treatment × time = NS
	6	6.36 ± 1.77	11.68 ± 1.78	
	24	6.73 ± 0.93	7.65 ± 0.93	
	48	7.07 ± 0.74	6.17 ± 0.74	

Values are presented as the mean ± standard error. Two-way ANOVA was determined using a least squares fit. NS = $P > 0.05$. *Significantly different mean values as determined by Dunnett's test with $\alpha = 0.05$ as compared to control at zero time (n = 4).

the carbon flux through pyruvate kinase by way of potentiating its allosteric activation by fructose-1,6-diphosphate (McCarty, 1999). This hypothesis would explain metformin's effect of reducing hepatic triglyceride synthesis and failure to induce hypoglycemia. But in chickens metformin-HCl does induce hypoglycemia, thus, discounting this premise for metformin action in birds.

A second study suggests that metformin acts through the activation of AMP-activated protein kinase (AMPK), which is a major cellular regulator of lipid and glucose metabolism (Zhou et al., 2001). Once AMPK is activated it proceeds to inactivate acetyl-CoA carboxylase, the proximal rate-limiting step in lipogenesis (Hardie and Carling, 1997). Also inhibitors of AMPK activity have been shown to block the ability of metformin to inhibit hepatic glucose production (Zhou et al., 2001). The AMPK has also been implicated in upregulating muscle glucose uptake. The effects observed on blood hormones and metabolites in this study support these proposed mechanisms. By metformin-HCl inactivating acetyl CoA carboxylase one would observe an increase in fatty acid oxidation in the liver followed by decreased plasma triglycerides. This reduction in plasma triglycerides would be followed by a compensatory response in glucose regulatory elements (insulin/glucagon).

The pattern of metabolic response by the chicken to metformin-HCl treatment is similar to that for mammals but its specific effects in birds requires further investiga-

tion. Measurement of lipogenic/lypolytic activities in the liver, coupled with the response of endogenous chicken AMPK may shed light on the mechanism of action of metformin in birds. Metformin-HCl may also be important in studying hypoglycemia in birds, a condition rarely observed and difficult to maintain. The impact of metformin-HCl on feeding behavior may also have commercial use as a novel appetite suppressant in birds.

ACKNOWLEDGMENTS

The authors would like to acknowledge Donna Brocht and Beverly Russell for their expert technical assistance in obtaining the results of this work. Mention of a trade name, proprietary product, or vendor does not constitute a guarantee or warranty of the product by USDA or imply its approval to the exclusion of other suitable products or vendors.

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